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# Tumor Necrosis Factor: An Apoptosis JuNKie?

Review

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**TNF's main function is to stimulate inflammation by turning on gene transcription through the IKK/NFκB and JNK/AP-1 signaling cascades. TNF also can trigger apoptosis through caspase-8, but the role and underlying mechanism of this activity are not fully understood. Here, we review recent data on the role of JNK in the regulation of TNF-dependent apoptosis and discuss what is known so far about how cells decide whether to live or die in response to TNF.**

## TNF as a Key Inducer of Proinflammatory Genes

Tumor necrosis factor-α (TNF) plays a pivotal role in orchestrating innate inflammatory responses in vertebrates. Upon detection of invading intracellular pathogens, tissue macrophages and T cells produce either membrane-associated TNF (mTNF) or proteolytically derived soluble TNF (sTNF). TNF triggers local expression of chemokines and cytokines, promoting the adhesion, extravasation, attraction, and activation of leukocytes at the site of infection. Later, TNF facilitates transition from innate to acquired immunity by enhancing antigen presentation and T cell costimulation.

TNF is the prototype of ~20 related cytokines that act through specific members of the TNF receptor (TNFR) superfamily, mainly to modulate immunity (reviewed in Locksley et al., 2001). TNF homologs exist in insects, primitive chordates, amphibians, fish, birds, and mammals. Mammalian TNF signals through two distinct cell-surface receptors: TNFR1, the primary receptor for sTNF, and TNFR2, the main receptor for mTNF (reviewed in Wajant et al., 2003). These receptors trigger several intracellular signaling pathways, including the I-kB kinase (IKK), c-Jun N-terminal kinase (JNK), and p38 or p42/44 mitogen-activated protein kinase (MAPK) cascades, which control gene expression through transcription factors such as NF-κB and AP-1.

Most cell types constitutively express TNFR1 while TNFR2 expression is highly regulated. TNFR1 and 2 resemble each other in their extracellular, cysteine-rich domains. TNFR1 contains a cytoplasmic death domain (DD) that binds to the adaptor TRADD (TNFR-associated DD). TNFR2 lacks a DD, but has a cytoplasmic motif that binds TRAFs (TNFR-associated factors). The homotrimeric TNF ligand binds a pre-associated receptor homotrimer, inducing conformational changes that enable the cytoplasmic motifs to bind cognate signaling adaptors (Locksley et al., 2001). Upon binding to ligated TNFR1, TRADD recruits the secondary adaptors RIP1, TRAF2, or TRAF5. This causes activation of the IKK complex, which consists of IKK α, β, and γ (also called Nemo), through an unknown mechanism (reviewed in Chen and Goeddel, 2002) (Figure 1). IKK stimulates NF-κB by catalyzing phosphorylation and degradation of the NF-κB inhibitor, I-κB (reviewed in Karin and Lin, 2002). TRAF2 functions also as an obligatory conduit for stimulation of JNK through its MAPK kinase MKK7, promoting phosphorylation of c-Jun and thus increasing AP-1 activity. Unlike TNFR1, TNFR2 binds TRAF2 directly, hence activating IKK and JNK (Figure 1). TRAF2 also recruits ancillary proteins that modulate signaling through each TNFR, i.e., cIAP (cellular inhibitor of apoptosis protein) 1 and 2, and TRAF1. cIAP1 supports ubiquitination and degradation of TRAF2, whereas TRAF1 inhibits TNFR2-dependent signaling through an unknown mechanism (Wajant et al., 2003).

## TNF as a Conditional Death Ligand Blocked by NF-κB

While TNF's cardinal role is to stimulate inflammation, it is capable also of inducing apoptosis when NF-κB signaling is blocked. The precise biological role of this activity is unclear. TNF may function alongside of "professional" death ligands such as FasL and Apo2L/TRAIL to help cytotoxic leukocytes kill pathogen-infected cells. Perhaps TNF's apoptotic capability contributes to its established pathological role in rheumatoid arthritis and inflammatory bowel disease.

The "intrinsic" apoptosis pathway—triggered by intracellular injury such as DNA damage—controls caspase activation through the Bcl-2 gene family (reviewed in Puthalakath and Strasser, 2002; Cory and Adams, 2002). In this pathway, damage sensors induce transcription of Bcl-2 homology 3 (BH3) domain proteins (e.g., Puma, Noxa, Bim, Bmf). These apical regulators activate downstream proapoptotic Bcl-2 relatives (e.g., Bax, Bak), overcoming inhibition by antiapoptotic Bcl-2 family members (e.g., Bcl-2, Bcl-$X_L$). The activated Bcl-2 relatives trigger mitochondrial release of factors that promote caspase activation in the cytosol. One factor is cytochrome c, which cooperates with Apaf-1 to activate caspase-9. This apical caspase activates the effector caspases 3, 6, and 7, causing apoptotic death. Two other mitochondrial factors, Smac/Diablo and Omi/HtrA2, prevent IAPs from inhibiting caspase activation.

The "extrinsic" pathway is triggered by extracellular death ligands such as the TNF relatives FasL and Apo2L/TRAIL, which signal respectively through Fas and DR4 or DR5 (reviewed in Ashkenazi, 2002). These death receptors bind directly to the adaptor FADD (Fas-associated DD), which mediates recruitment and activation of caspases-8 and -10 within a death-inducing signaling complex (DISC). Caspases-8 and -10 activate apoptotic death through the same effector caspases as the intrinsic pathway. Modulation of the extrinsic pathway occurs at several levels. Decoy receptors can compete with death receptors for ligand binding. The caspase-related molecule c-FLIP (cellular FLICE-inhibitory protein), which lacks catalytic activity, competes with caspases-8 and -10 for DISC binding (reviewed in Thome and Tschopp, 2001). Further downstream, IAPs inhibit

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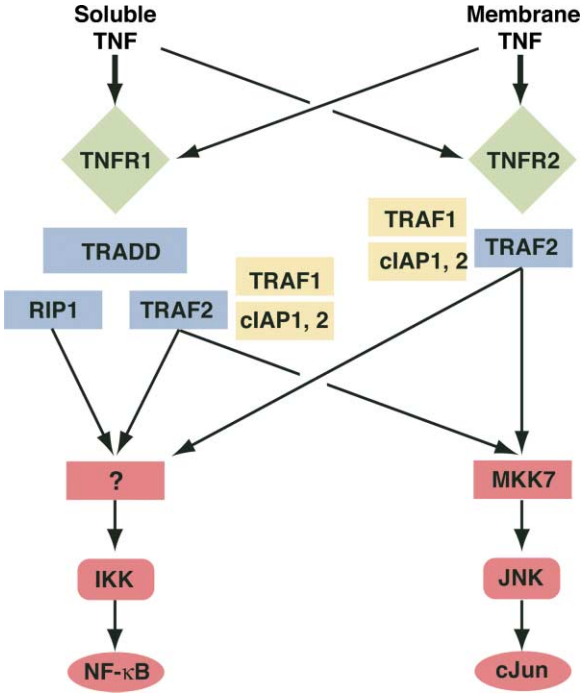


Figure 1. Model for Control of Gene Transcription by TNF

effector caspase activation (reviewed in Salvesen and Duckett, 2002). In "type I cells," the DISC generates sufficient caspase activity to trigger death. In "type II cells," apical caspase activation is weaker, and apoptosis requires amplification through crosstalk to the intrinsic pathway: caspase-8 cleaves the BH3 protein Bid, which stimulates Bax and Bak to augment caspase activation (reviewed in Peter and Krammer, 2003).

The pleiotropic nature of TNF has hindered elucidation of its apoptosis signaling mechanism. TNF does not usually trigger apoptosis in TNFR-bearing cells. However, general inhibition of transcription or translation or selective blockade of the IKK/NF-κB pathway uncovers TNF's proapoptotic capacity (Chen and Goeddel, 2002; Karin and Lin, 2002). Although mice with *TNF* or *TNFR* gene knockouts develop normally, mice deficient in NF-κB signaling die in utero from TNF-dependent apoptosis of liver cells. By activating NF-κB, TNF induces a number of antiapoptotic genes, including *c-FLIP*, *cIAP1*, *cIAP2*, *A1*, *A20*, *TRAF1*, and *TRAF2*.

## TNFR1 and 2 as Mediators of TNF-Induced Apoptosis

Challenge of mice with bacterial lipopolysaccharide (LPS) together with the liver-specific transcription inhibitor D-galactosamine (GalN) stimulates systemic release of sTNF, which induces hepatocyte apoptosis and liver failure. TNFR1-deficient mice are resistant to this effect, while TNFR2 knockouts are sensitive. Thus, sTNF signals hepatocyte apoptosis mainly through TNFR1. Indeed, TNF-deficient mice expressing a transgenic, noncleavable mTNF mutant are largely resistant to LPS/GalN challenge. Unlike LPS, the T cell stimulator Concanavalin A (ConA) induces mostly mTNF. ConA causes liver damage that requires both TNFR1 and 2 for maximal apoptosis. Thus, mTNF uses both receptors to trigger hepatocyte death (Maeda et al., 2003 and references therein).

TNFR1 shares several components of the extrinsic death pathway with Fas, DR4, and DR5. Mouse embryo fibroblasts (MEFs) deficient in either FADD or caspase-8 resist TNF-induced apoptosis, demonstrating an obligatory role for these molecules. Previous studies with transfected cells suggested that TNFR1 assembles a DISC similar to that of Fas and DR4/5, except that this occurs indirectly through TRADD (Chen and Goeddel, 2002). A recent study examined a wild-type HT1080 human fibroblast cell line, resistant to sTNF-induced apoptosis, and a mutant line with defective NF-κB activation, sensitive to TNF killing (Micheau and Tschopp, 2003). In both lines, sTNF induces TRADD-mediated assembly of a TNFR1-associated complex (complex I) that contains RIP1, TRAF2, and cIAP1 and activates the IKK/NF-κB pathway (Figure 2). Subsequently, TRADD, RIP1, and TRAF2 undergo biochemical modifications and the complex dissociates from TNFR1, moving to the cytosol. FADD and caspase-8 bind to this cytosolic complex (complex II). In wild-type cells, complex II contains abundant c-FLIP but little caspase-10, while in mutant cells the converse is true. Thus, c-FLIP may be an important NF-κB-dependent factor preventing apical caspase activation at the level of complex II, possibly by competing for caspase-10 binding. Besides c-FLIP, cIAP1 and TRAF1 are more abundant in complex II of the mutant cells and might inhibit caspase activation (Micheau and Tschopp, 2003; Wang et al., 1998).

Simultaneous engagement of both TNFRs amplifies TNF-induced apoptosis (Wajant et al., 2003; Maeda et al., 2003). This correlates with increased TNFR2-induced TRAF2 degradation. Since TRAF2 recruits cIAPs to TNFR1, its degradation via TNFR2 may facilitate cell death. TRAF2 destruction also attenuates TNFR1-mediated NF-κB activation, further promoting apoptosis. Thus, TRAF2 may provide an additional switch between inflammation and cell death downstream of TNF.

## JNK as a Regulator of Apoptosis

JNK1, 2, and 3 (also known as stress-activated protein kinases) form a subgroup of the MAPK superfamily that is activated by cell stressors such as ultraviolet (UV) radiation and by proinflammatory cytokines such as TNF and interleukin-1 (reviewed in Weston and Davis, 2002; Shaulian and Karin, 2002; Lin, 2003). JNK phosphorylates specific subunits, namely c-Jun, JunB, JunD, and ATF-2, of the AP-1 transcription factor, turning on genes that control diverse cellular functions including proliferation, differentiation, and apoptosis. JNK's precise role in apoptosis remains controversial since it appears to have conflicting effects depending on the species, type of cell, or nature of death stimulus.

*Drosophila melanogaster* has counterparts to the major components of the mammalian JNK cascade as well as orthologs of many mammalian cell death genes. In *Drosophila*, apoptosis during embryonic patterning of the wing, eye, and gut requires the fly's JNK ortholog *DJNK* (also called *Basket*) (Kockel et al., 2001; Moreno et al., 2002; and references therein) (Figure 3). Ectopic

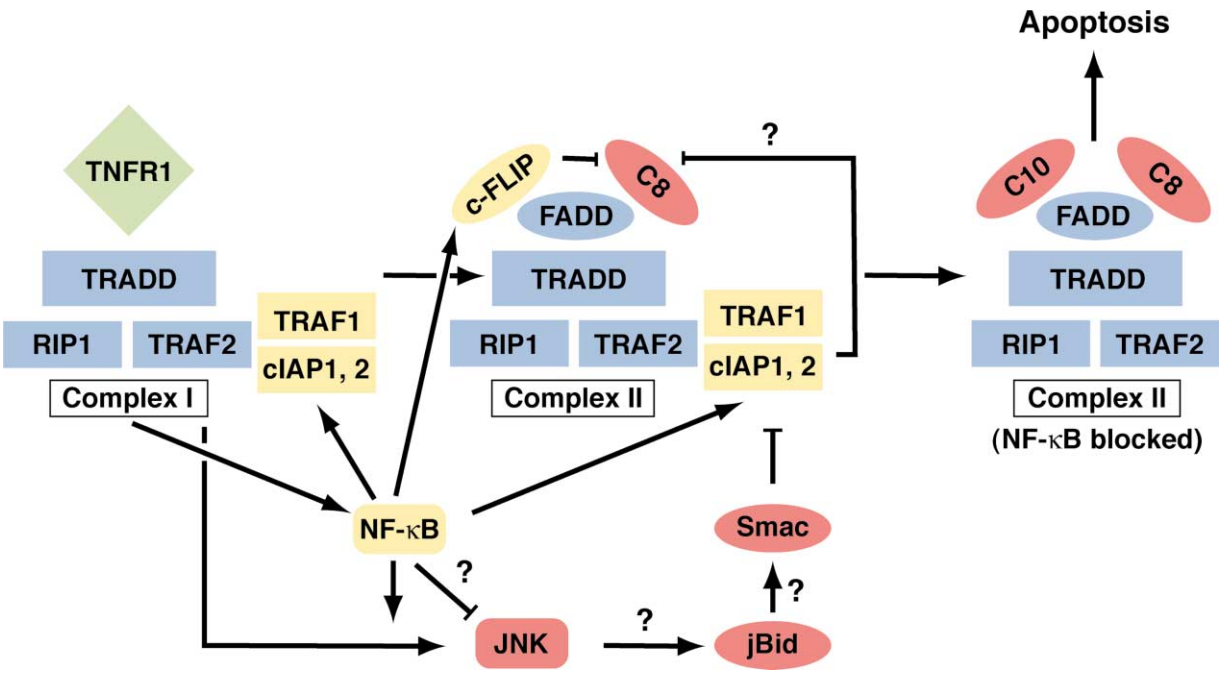


Figure 2. Model for Apoptosis Control by TNFR1

DJNK activation in the eye imaginal disc causes excessive cell death and eye ablation. This phenotype involves DJNK-dependent phosphorylation of DJun, which promotes transcription of *Hid* and *Rpr*. Hid, Rpr, and another fly gene called *Grim* encode proteins with a related sequence motif that enables them to induce cell death by binding to the fly IAP ortholog DIAP1. This binding prevents DIAP1 from blocking activation of the fly caspase DRONC, much like the interaction of mammalian Smac and IAPs (Salvesen and Duckett, 2002). DIAP1 also attenuates DJNK activation by promoting degradation of DTRAF1, the fly ortholog of mammalian TRAF2. DJun turns on an additional negative-regulatory feedback loop by promoting transcription of *Puckered* (*Puc*), a dual specificity phosphatase that inactivates DJNK. Thus, in *Drosophila*, JNK plays a crucial role in a tightly regulated signaling pathway that promotes apoptosis.

In mammals, there is evidence both for proapoptotic and for antiapoptotic JNK activity (Lin, 2003 and references therein). Apoptotic death of rat PC12 neuronal cells deprived of nerve growth factor (NGF) requires JNK activity. Knockout of *JNK1* and *JNK2* in the mouse suppresses apoptosis in the hindbrain neuroepithelium at day 9.25, but causes increased apoptosis in the hindbrain and forebrain at day 10.5. *JNK1*/*JNK2*-deficient MEFs resist apoptosis induction by UV radiation, proteasome inhibitors, or genotoxic drugs. Furthermore, in *JNK1* or *JNK2* knockout mice, thymocytes are refractory to death in response to T cell receptor ligation, while in *JNK3*-deficient mice, hippocampal neurons resist apoptosis induction by excitotoxic stress. JNK may promote mammalian cell apoptosis by engaging the cell-intrinsic pathway (Figure 4). Whereas wild-type MEFs die in response to UV or as a result of ectopic expression of a

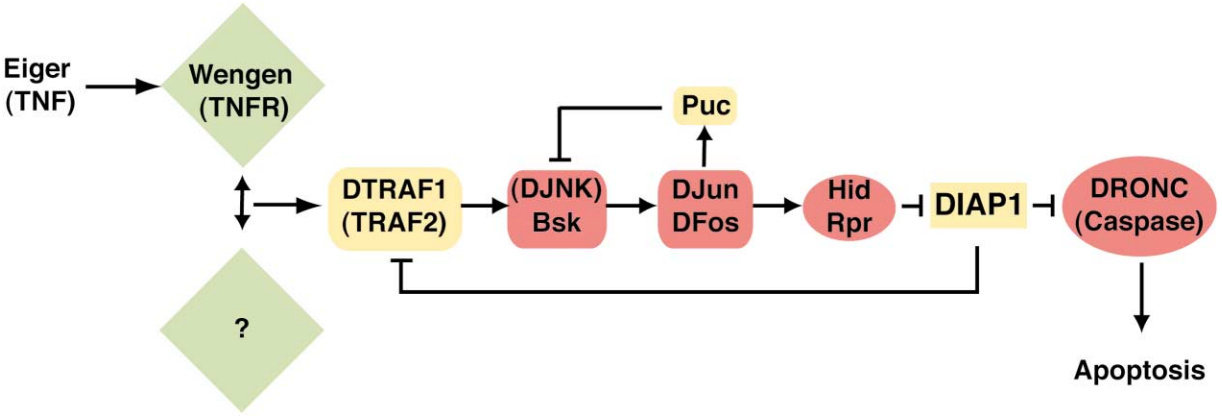


Figure 3. Model for Apoptosis Control by *Drosophila* Eiger

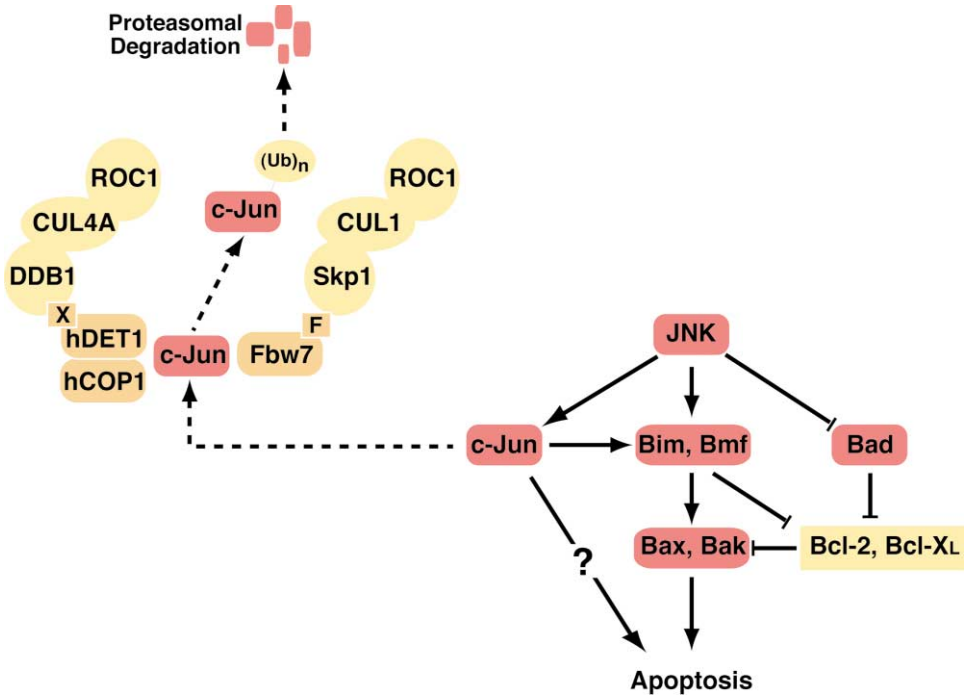


Figure 4. Model for Mammalian Apoptosis Modulation by JNK

constitutively active MKK7-JNK fusion protein, *Bax/Bak* knockout MEFs do not (Lei et al., 2002). Moreover, JNK1/*JNK2*-deficient MEFs fail to show Bax activation, cytochrome c release, or death upon UV exposure. JNK-dependent phosporylation of the BH3 proteins Bim and Bmf causes their dissociation from dynein and myosin motor complexes, as does UV radiation; this may free Bim and Bmf to activate apoptosis through Bax and Bak (Lei and Davis 2003 and references therein). JNK also promotes Bim transcription through c-Jun (Shaulian and Karin, 2002).

In contrast to the proapoptotic activity of JNK in NGF-deprived PC12 cells, new data suggest that JNK mediates pro-survival signals downstream of interleukin-3 (IL-3) in human FL5.12 pro-B cells (Yu et al., 2004). IL-3, a crucial survival factor for FL5.12 cells, stimulates JNK, while its withdrawal decreases JNK activity. Inhibition of JNK with the low molecular weight compound SP600125 partially attenuates apoptosis of FL5.12 cells after IL-3 withdrawal, whereas expression of a constitutively active JNKK2-JNK1 fusion protein promotes this response. Thus, JNK contributes to survival signaling in FL5.12 cells downstream of IL-3. Earlier work showed that IL-3 promotes phosphorylation of the BH3 protein Bad predominantly on serine residues (Ser122 and 136), and that this phosphorylation mediates the survival signal of IL-3 in FL5.12 cells; current work shows that activated JNK phosphorylates Bad on threonine 201; this inhibits Bad's association with Bcl-$X_L$, probably freeing up more Bcl-$X_L$ to block apoptosis (Yu et al., 2004 and references therein). These findings suggest a novel mechanism for JNK-dependent inhibition of cell death (Figure 4). However, the prevalence of Bad phosphorylation on threonine 201 by JNK, as compared to phosphorylation on serines by other kinases that may promote survival downstream of IL-3, e.g., Akt, remains to be determined.

### JNK as a Contextual Modulator of TNF-Induced Apoptosis

While there is experimental evidence that JNK can inhibit apoptosis induction by TNF, other data suggest that it can function as an important positive regulator of TNF-induced apoptosis. In *Drosophila,* a homolog of the mammalian TNF superfamily called Eiger stimulates apoptosis through a *JNK*-dependent mechanism (Igaki et al., 2002; Moreno et al., 2002; and references therein) (Figure 3). Eiger binds to a TNFR-related fly protein called Wengen (Kanda et al., 2002). Although Wengen is required for Eiger activity, it does not possess recognizable binding motifs for signaling adaptors such as TRAFs or TRADD, nor does it display detectable signaling function. Hence, Wengen may be a ligand binding subunit of a more complex signaling receptor. Regardless, Eigen acts through Wengen to induce DTRAF1-dependent stimulation of DJNK, thereby inducing apoptosis. As with *DJNK*, ectopic *Eiger* expression in the eye results in excessive apoptosis and eye ablation, and this phenotype can be suppressed by *Puc*.

Several studies in mice demonstrate an inhibitory role for JNK in TNF-induced cell death. *TRAF2* knockout MEFs, which have largely intact NF-κB signaling but reduced JNK stimulation, display increased apoptosis sensitivity to TNF (Wajant et al., 2003). The JNK inhibitor SP600125 enhances TNF-induced apoptosis of MEFs deficient in the *RelA* subunit of NF-κB. Furthermore, MEFs deficient in *JNK1* and *JNK2* show increased sensitivity to TNF killing, and their transfection with *JNK1* or *JunD* rescues TNF resistance (Lamb et al., 2003 and references therein). Conversely, *JNK2*-deficient MEFs,

isolated from a different mouse genetic background than the latter MEFs, display a moderate resistance rather than sensitization to TNF killing (Dietrich et al., 2003). Moreover, constitutive JNK1/2 activation in cells with deficient NF-κB signaling sensitizes to TNF-induced apoptosis (Lin, 2003). *JNK1* and *JNK2* knockouts are much less sensitive than wild-type mice to ConA-induced liver damage (which involves mTNF); furthermore, SP600125 blocks TNF-induced death of *IKK*β-deficient mouse hepatocytes (Maeda et al., 2003).

JNK's involvement in TNF-induced apoptosis may depend on NF-κB. TNF activates JNK transiently; however, in cells with general inhibition of transcription or specific inhibition of NF-κB, TNF leads instead to prolonged JNK activation (Lin, 2003; Maeda et al., 2003). The NF-κB-dependent genes *A20*, *GADD45*β, and *XIAP* attenuate TNF activation of JNK upon ectopic expression (Lin, 2003). However, *A20*-deficient MEFs, which display increased sensitivity to TNF-induced death, have normal JNK activation (Lee et al., 2000). Similarly, *GADD45*β-deficient MEFs or mouse splenocytes show unaltered JNK activation or apoptosis induction by TNF plus cycloheximide, although this result has been challenged (Amanullah et al., 2003). While *XIAP* knockout mice develop normally (Harlin et al., 2001), sensitivity of *XIAP*-deficient cells to TNF stimulation of JNK or apoptosis has yet to be analyzed. Thus, the specific mechanisms by which NF-κB limits the duration of JNK activation remain obscure.

A recent study suggests that JNK may play an essential positive role in TNF-induced apoptosis (Deng et al., 2003). In *RelA*-deficient MEFs or human HeLa cells expressing an *I-κB* mutant that blocks NF-κB, small interfering RNA (siRNA) knockdown of MKK7 prevented TNF-induced caspase-8 processing and cell death. TNF stimulated an MKK7-dependent (and presumably, JNK-dependent) cleavage of the BH3 protein Bid. MKK7/JNK-mediated Bid processing generated a unique product (termed jBid), distinct from the previously identified product of Bid cleavage by caspase-8. A Bid deletion mutant similar to jBid in size translocated to the mitochondria, where it selectively triggered the release of Smac, but not of cytochrome c. SiRNA knockdown of Smac prevented TNF from inducing caspase-8 processing and apoptosis, while transfection of activated Smac augmented these TNF effects, suggesting that Smac acts upstream of caspase-8 activation. A Smac-based peptide, known from other studies to block IAP interactions with caspases, inhibited association of cIAP1 and TRAF2. These data suggest a model in which TNF activates caspase-8 by relieving it from cIAP inhibition through MKK7/JNK-dependent jBid generation and consequent Smac release (Figure 2). However, despite evidence for inhibitory association of cIAPs with caspases-3, -7, or -9, support for interaction with caspase-8 (or 10) is lacking (Salvesen and Puckett, 2002). Moreover, overexpression of cIAP1 and 2 in HT1080 cells expressing mutant I-κB did not protect against TNF killing (Wajant et al., 2003). So it remains unclear how jBid promotes caspase-8 activation through Smac. The jBid model raises several other intriguing questions: (1) How does MKK7/JNK activation generate jBid? Is transcription required? (2) Do inhibitors of caspases or other proteases block jBid generation? (3) What is jBid's N-terminal structure? (4) How does jBid selectively release mitochondrial Smac without affecting cytochrome c? (5) Does ectopic expression of jBid inhibit the association of cIAP1 with TRAF2 within complex II? (6) Is jBid involved in TNF-induced apoptosis in type I as well as type II cells?

Besides acting at the level of direct or indirect post-transcriptional modification of death signaling molecules, JNK may modulate apoptosis through AP-1-dependent gene transcription. This is exemplified by JNK's regulation of Bim mRNA levels through c-Jun (Shaulian and Karin, 2002). The level of c-Jun in cells is controlled by ubiquitination and consequent proteasomal degradation, but the underlying enzymatic machinery has been elusive. New work uncovers two specific ubiquitin ligases that support c-Jun destruction in neuronal and nonneuronal cells, affecting AP-1 activity as well as apoptosis (Nateri et al., 2004; Wertz et al., 2004; and references therein) (Figure 4). Numerous mammalian F box proteins function as substrate adaptors for ubiquitin ligases of the SCF (Skp1/Cullin/F box protein) type. A yeast two-hybrid screen of a brain cDNA library, designed to identify specific binders of phosphorylated c-Jun, detected the F box protein Fbw7 (Nateri et al., 2004). Fbw7 bound to phosphorylated c-Jun and promoted its ubiquitination, but did not interact with a phosphorylation-defective c-Jun mutant or with ATF2. Ectopic expression of Fbw7 and c-Jun in 293T human embryonic kidney cells induced proteasomal degradation of phosphorylated c-Jun, attenuating AP-1 activity. Conversely, siRNA knockdown of Fbw7 in rat PC12 cells led to accumulation of phospho-c-Jun, increasing AP-1 activity. Importantly, Fbw7 depletion in PC12 cells elevated basal apoptosis levels and substantially augmented apoptosis induced by NGF deprivation. Expression of the JNK-inhibitory scaffolding protein JIP-1 reversed these proapoptotic effects, indicating that they are required for JNK activity. Fbw7 depletion also augmented apoptosis of mouse primary cerebellar neurons in a c-Jun-dependent fashion. Thus, in neuronal cells, modulation of c-Jun levels by the $SCF^{Fbw7}$ ubiquitin ligase complex appears to provide an important mechanism for controlling JNK-dependent apoptosis (Nateri et al., 2004).

Different ubiquitin ligase complexes may control c-Jun degradation in other tissues. Indeed, pulldown experiments with hDET1 (the human homolog of de-Etiolated 1, an *Arabidopsis thaliana* protein that regulates plant photomorphogenesis), identified a distinct ubiquitin ligase complex that regulates c-Jun ubiquitination in 293T epithelial cells and in U2OS osteosarcoma cells (Wertz et al., 2004) (Figure 4). hDET1 binds to hCOP1, a human homolog of *Arabidopsis Constitutively Photomorphogenic-1*, together forming a ubiquitin ligase substrate adaptor that brings c-Jun into contact with a ubiquitin ligase complex that consists of three proteins: DNA damage binding protein 1 (DDB1), Cullin 4A (CUL4A), and Regulator of Cullins-1 (ROC1). Like Fbw7, hCOP1 possesses WD40 repeats; however, neither hDET1 nor hCOP1 contains an F box, suggesting that an unidentified "X box" mediates binding of the hDET1/hCOP1 heterodimer to the rest of the complex (Figure 4). This ligase complex, termed $DCX^{hDET1/hCOP1}$, catalyzed ubiquitination of c-Jun in vitro and in 293T

and U2OS cells. Ectopic coexpression of *hCOP1* and *hDET1* led to proteasome-mediated c-Jun degradation, and siRNA knockdown of hDET1 led to c-Jun accumulation, increasing AP-1 activity and apoptosis. Thus, in nonneuronal cells, modulation of c-Jun levels by the $DCX^{hDET1/hCOP1}$ complex provides an important mechanism for controlling JNK-dependent apoptosis, similar to the action of $SCF^{Fbw7}$ in neuronal cells. Given the extensive diversity of the ubiquitin-proteasomal system, it is likely that multiple ubiquitin ligases regulate JNK-dependent apoptosis in various cell types at the level of c-Jun and perhaps of other AP-1 subunits. These new findings raise the intriguing, as yet untested hypothesis that ubiquitin ligase activity against c-Jun may regulate JNK's contribution to TNF-induced apoptosis. If so, then a logical question that follows is whether NF-κB uses this mechanism to exert additional inhibitory control over TNF-induced cell death, perhaps by promoting the expression of specific ubiquitin ligase components.

## Conclusions

TNF's primary role is to stimulate inflammatory cells to fight infection, whereas the function of its proapoptotic capability remains mysterious. Nonetheless, it makes sense that the main conduit for TNF's proinflammatory action, NF-κB, strongly inhibits TNF's induction of apoptosis. TNF turns on several antiapoptotic genes through NF-κB. Notable amongst those is c-FLIP, which blocks apical caspase activation in a cytosolic DISC that forms downstream of TNFR1. NF-κB stimulation also limits the duration of TNF-induced JNK activity; conversely, NF-κB inhibition is associated with prolonged TNF-induced JNK activation and apoptosis. How NF-κB controls JNK activity is not yet clear, though several NF-κB-induced genes have been implicated.

New evidence supports a positive, if not essential, role of JNK in TNF-induced apoptosis. In *Drosophila*, the TNF superfamily homolog Eiger requires *DJNK* to induce cell death. However, Eiger engages the DRONC caspase independently of DFADD and the *Drosophila* caspase-8 homolog DREDD, which in the fly regulate inflammation rather than apoptosis. In contrast, mammalian TNF requires TRADD, FADD, and caspase-8 for apoptosis induction, and JNK may modulate this by promoting caspase-8 activation. In cells with blocked NF-κB, JNK may trigger an unusual path to caspase-8 activation, involving a novel modification of Bid. Future work should define biochemically how JNK supports Bid processing and caspase-8 activation.

Both in neuronal and nonneuronal cells, newly identified ubiquitin ligase complexes that support proteasomal degradation of c-Jun reveal a novel mechanism for control of JNK-associated apoptosis. It will be interesting to examine whether this mechanism also modulates JNK's contribution to TNF-induced apoptosis, and if so, whether NF-κB affects the transcription of specific ubiquitin ligase components. The broader question of how cells integrate NF-κB-controlled determinants with other signals downstream of TNF is ripe for the emerging field of systems biology. Indeed, the first example for such exploration of TNF/NF-κB signaling has just been reported (Bouwmeester et al., 2004). Understanding how cells choose life or death after TNF stimulation will undoubtedly shed new light on the role of TNF-induced apoptosis in health and disease.

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